Morphological, Ultrastructural and Physiological Characteristics of Damage to an Extensive Stand of the Lichen *Usnea sphacelata* at Casey Station, East Antarctica

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An extensive stand of the foliose lichen, *Usnea sphacelata*, in the immediate vicinity of the new Casey Station (lat. 66°17′S, long. 110°32′E), East Antarctica, displays unusual symptoms of severe damage, the cause of which is unknown. The heavily pigmented upper sections of the thallus are absent giving it the appearance of having been uniformly shaved and the remnant tips are frequently bleached with the central strand often protruding beyond the remaining medulla and outer cortex. Where damage has occurred well above the holdfast, the lower unpigmented sections of the thallus often remain comparatively healthy. If damage occurs closer to the base, only stumps of the holdfast remain. Affected lichens are located in two discrete patches characterized by very sharp boundaries with surrounding healthy material of the same species. This study; (i) provides a description of the damaged site, (ii) documents morphological and ultrastructural features of the damage and (iii) compares the photosynthetic pigment composition and chlorophyll fluorescence characteristics of damaged and healthy specimens. Despite massive pruning of the foliose *Usnea* thallus and substantial bleaching of the remaining basal portions (around 80% chlorophyll and carotenoid loss), the results indicate that many of the damaged lichens contain viable algal cells. New shoots are plentiful among many of the remaining lichen stumps. We therefore suggest that the damaged areas described in this paper have the potential for substantial recovery.

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INTRODUCTION

Casey (lat. 66°17′S, long. 110°32′E) is a permanently occupied Australian research station located in the Windmill Islands region of Wilkes Land, East Antarctica (Fig. 1). The northern half of the Windmill Islands region is characterized by low lying rocky outcrops of the Windmill Metamorphics group, a layered sequence of schists, gneisses and migmatites (Blight and Oliver, 1982), separated by areas of permanent snow and ice. The southern half of the region is dominated by the Ardery Charnockite intrusion, with a small area of porphyritic granite (Blight and Oliver, 1982). Glacial erratic boulders and stones, of predominantly metamorphic origin, are widely distributed.

The region supports an abundant and extensive cryptogamic floral community consisting of at least five moss, one hepatic and more than twenty eight lichen taxa (Lewis-Smith, 1986a, 1988; Seppelt, unpublished data). At least ninety soil, snow and freshwater algal taxa are currently being investigated (Ling, 1990, pers. comm). Exposed rock, loose stones and gravel around the new station site and elsewhere within the region support extensive stands of lichen, of which *Usnea sphacelata* R. Br., *Usnea antarctica* Du Rietz and *Umbilicaria decussata* (Vill.) Zahlbr. are dominant species.

The hill immediately to the south-east of the new station domestic building is well vegetated, but extensive areas of lichen on its north facing slope are seriously damaged. This site was first described by Lewis-Smith (1986b) with the brief statement; 'On this hillside, a dense stand of the lichen *Usnea sphacelata* (= *U. sulphurea*), covering 2 ha, has been almost completely killed by some form of pollution.' He suggested that the most likely cause of damage was contamination by cement dust released during construction of the new station. This area is dominated by *U. sphacelata* with examples of damaged *U. antarctica*, *U. decussata* and *Pseudephebe minuscula* (Nyl. ex. Arnold) Brodo and Hawksworth also present. The most obvious symptom of damage at this site is the loss of the black upper branches of the *U. sphacelata* thalli, leaving only the pale basal sections attached to the rocks and gravel of the substrate.

This study was carried out to obtain baseline data for assessing future vegetation trends, that is, to monitor recovery or further deterioration of the damaged areas. It has four specific aims: to describe the location and extent of the lichen damage described above; to record the symptoms of damage exhibited by the dominant species *U. sphacelata*; to assess the capacity of physically damaged plants for photosynthesis and to compare the findings with those obtained in a similar investigation of airborne alkaline pollution damage to lichens growing downwind of the concrete batching site at Casey (Adamson and Seppelt, 1990).

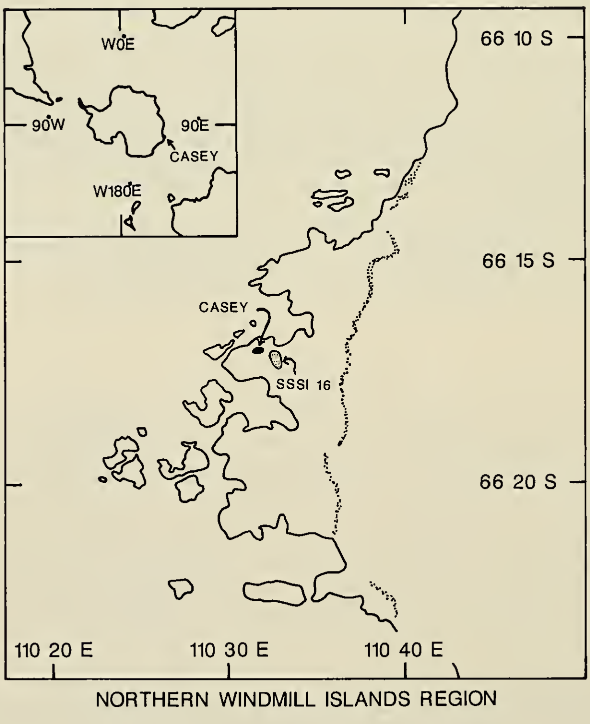

Fig. 1. Location map of Casey Station and the Site of Special Scientific Interest number 16 (S.S.S.I. 16) within the northern Windmill Islands region.

Methods

Preliminary investigations of the morphological features, chlorophyll and carotenoid content and photosynthetic capacity of healthy and damaged *U. sphacelata* were carried out in the field and laboratory at Casey during the summers of 1988-89 and 1989-90. Specimens were also returned to Australia for detailed analysis. They were frozen immediately after collection and kept at -20°C until use. The healthy specimens used for comparative purposes were located in an unaffected control area, 0.5 km to the east of the damaged site, in the Site of Special Scientific Interest number 16 (S.S.S.I. 16).

Pigment Analyses

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted into 80% acetone and their concentrations determined using standard spectrophotometric procedures. The control samples were trimmed such that only the lower stem, within 1-2 cm of the holdfast, was compared with samples from the damaged site. Pigment extractions were performed by grinding samples with acid washed sand, 50 mg magnesium carbonate and 10 ml acetone (80%) on ice in dim light. The slurry was transferred to a 10 ml centrifuge tube, shaken vigorously and spun at 2000 rpm for five minutes. The supernatant was then decanted, kept cold and dark, and the pellet resuspended in 1.5 ml acetone (80%) and centrifuged as before. The supernatants were then combined, made up to a known volume and analysed using a scanning spectrophotometer (Hitachi U3200) zeroed at 750 nm. The chlorophyll content was calculated from absorbance values at 663 and 645 nm, according to the equations of Anderson and Boardman (1964) as modified by Brouers and Michel-Wolwertz (1983). The total carotenoid content (per gram dry weight) was calculated from absorbance values at 480 and 510 nm according to Parsons *et al.* (1984). Results are expressed per gram dry weight, calculated from carefully matched samples dried at 80°C for 24 hours.

Fluorescence Measurement

The photosynthetic competence of the algal symbiont, *Trebouxia* sp., in selected representative lichens from both the damaged and control sites was determined using a non-destructive, rapid, chlorophyll fluorescence technique. Comparative field measurements of fluorescence characteristics of damaged and healthy thalli were made using a Branker Plant Productivity Meter (summer 1988-89) and a Biomonitor Plant Stress Meter, S.C.I., AB, Sweden (summer 1989-90). This was followed with further Plant Stress Meter measurements in Australia on frozen material which had been allowed to thaw at 5°C in dim light under moist conditions, for five days. For a technical description of this instrument see Oquist and Wass (1988). The actinic excitation light intensity was set at 300 μmol. m^{-2} sec^{-1} for a period of 10 seconds. A dark adaption time of fifteen minutes was allowed before measurements of initial (Fo) maximum (Fm) and variable (Fv) fluorescence were performed in darkness at 5°C. The principles and techniques of chlorophyll estimation by absorbance spectroscopy and chlorophyll fluorescence analysis are described by Hipkins and Baker (1986) and Lawlor (1987).

Scanning Electron Microscopy

Desiccated specimens were mounted on aluminimum stubs and secured using double sided sticky tape and carbon paint. They were coated with gold using a Polaren E5000 sputter coating unit and viewed in a JEOL JSM 840 scanning electron microscope (SEM). Alternatively, samples were mounted on aluminium tape, sputter coated with carbon and examined in a Philips 505 SEM.

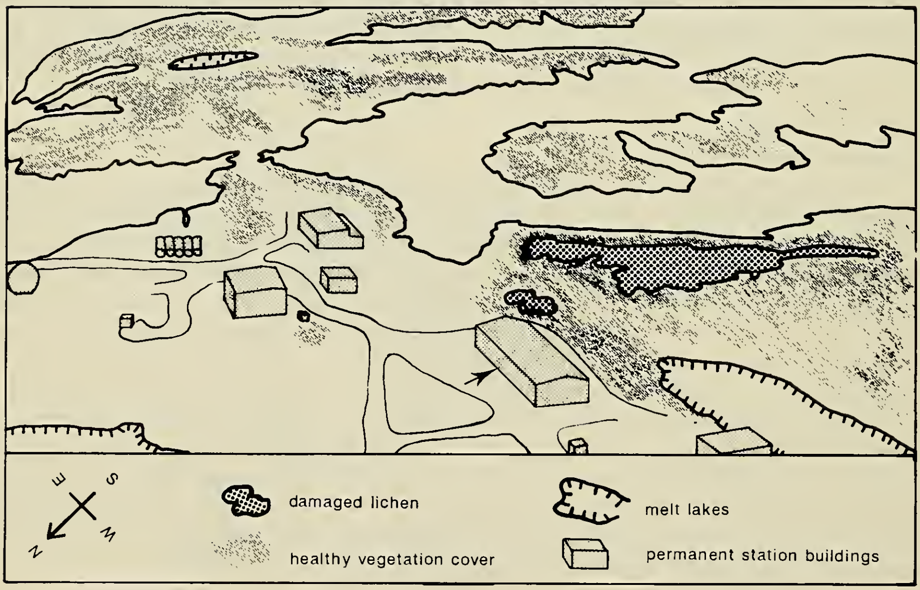

Fig. 2. Casey Station and adjacent areas. (**A**) Oblique aerial photograph showing the two areas of damaged lichen in relation to surrounding healthy vegetation and the eastern half of Casey station. S.S.S.I. 16 includes the well vegetated rocky hills at the top of the photograph. (**B**) Interpretation of the oblique aerial photograph highlighting the two areas of damaged lichen and the extent of surrounding healthy vegetation. Due to foreshortening caused by the oblique nature of the photograph, a scale is not given, but the long axis of the larger patch of damaged lichen is 139 m in length, and the long axis of the smaller damaged patch is 30.5 m in length. Arrow indicates new domestic building.

Transmission Electron Microscopy

Selected thalli were taken from -20°C, placed on moist filter paper in a petri dish and allowed to rehydrate for one hour at 4°C. The thalli were cut into 2-3 mm lengths and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at 4°C for 18 hours, washed thoroughly in the buffer and postfixed in buffered 1% OsO_4 for 2 hours at room temperature. The samples were dehydrated in a graded acetone series and embedded in a low viscosity epoxy resin. Thin sections were stained sequentially in uranyl acetate and lead citrate and examined in a Philips 400 transmission electron microscope (TEM).

Soil pH

Soil pH was determined at the larger affected site for comparison with soil samples from S.S.S.I. 16 and other unaffected sites further from the station. Soil was collected to a maximum depth of 3 cm, dried and sieved through a 2 mm pore size soil sieve. 10 g of dry soil was then mixed with 50 ml ultrapure water (Milli-Q filter, Millipore) agitated on an orbital shaker for 10-15 minutes and pH measurement of the soil water slurry made with plastic pH strips (Merck Universal Indikator). A comparison using a portable pH meter (TPS LC 80) indicated that this method underestimates soil pH values by 0.28 +/- 0.08 pH units.

RESULTS

Descripton of the Affected Site

There are two distinct areas of apparenty bleached *Usnea* lichen which have lost the upper sections of their thalli (Figs 2**A**, **B**). Both are characterized by sharp but irregular boundaries with surrounding healthy lichen. The larger of the two areas covers approximately 6600 m^2 on the north facing side of the hill located immediately to the south-east of the new domestic building. This area is composed of an extensive slope of predominantly small stones, with scattered larger boulders. The damaged lichen occurs on both small and large rocks. The smaller affected area is approximately 360 m^2, dominated by large boulders and located on relatively level ground at the base of the affected slope. The two patches are separated by approximately 25 metres of similar rocky terrain vegetated with undamaged lichen and moss. Within each affected area, there are scattered small patches that appear unscathed. These often, but not exclusively, occur in sheltered areas in the lee of rocks and hollows. There may be some correlation between the location of these apparently healthy patches and snowdrift patterns or protection from the strong, predominantly easterly winds. The removal of the black upper sections of the damaged *U. sphacelata* makes the affected areas quite obvious. In the oblique aerial photograph (Fig. 2**A**) they can be clearly distinguished as pale patches surrounded by black areas of healthy lichen. These sites are not subject to vehicular or extensive foot traffic and do not appear to have been physically disturbed. Soil pH at the larger of the two affected sites was found to be 4.4, consistent with the regional mean of pH 4.

Symptoms of Damage to U. sphacelata

A comparison of typical healthy and damaged specimens of *U. sphacelata* is shown in Fig. 3. In the damaged specimens, the heavily pigmented upper parts of the thalli are missing, the remaining lower sections are somewhat bleached near the shear zone and the central strand frequently protrudes beyond the remaining medulla and cortex. The length of the remaining lower sections varies, but in most cases, the upper branches appear to have been sheared off at a uniform level (Figs 3, 4**A**, **B**). Where the holdfast

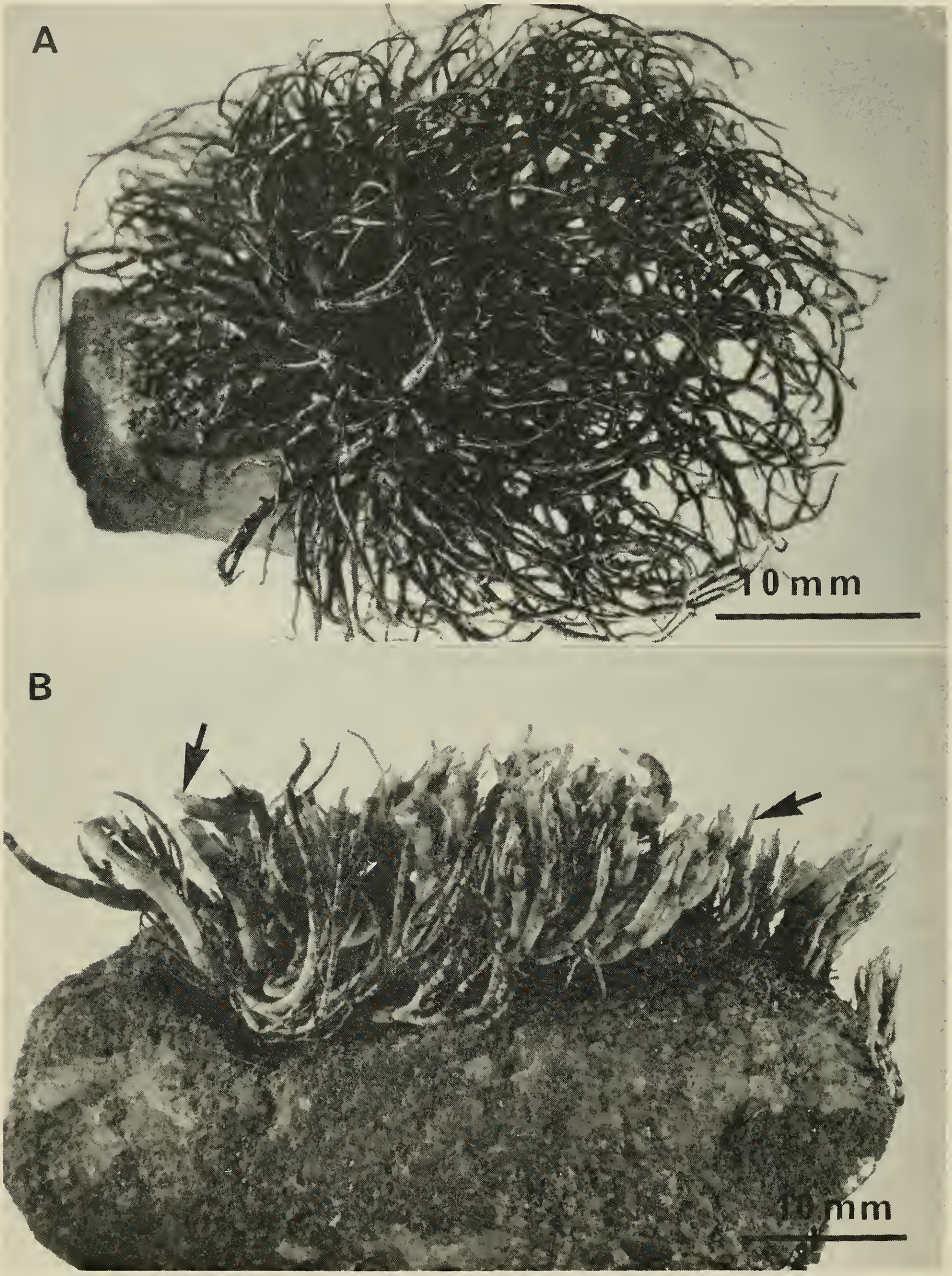

Fig. 3. Typical examples of (**A**) healthy and (**B**) damaged *U. sphacelata* from the S.S.S.I. 16 control site and the larger of the two damaged areas to the south-east of the new station domestic building. Note the heavily pigmented branching thallus of the healthy sample compared to the 'shaved' remnant thallus of the damaged sample with its upper branches missing and the central strand frequently protruding.

and lower sections of the lichen are growing from the underside of small rocks on the extensive gravel slope, the shear zone frequently corresponds with the level at which the lichen appears above the protection of surrounding rocks. The lower sections often appear to remain comparatively healthy although the cortex may split (Fig. 4C). In many samples, the shear zone occurs well above small intact branches in the region of the holdfast. These do not seem to be damaged and some may represent growth subsequent to the damage that has affected the rest of the lichen. It is also possible for one section of a lichen to be severely damaged while a significant portion appears totally unaffected. In the majority of cases, the holdfast and lower branches remain firmly attached. Similar symptoms of mild damage have occasionally been observed in isolated samples of *U. sphacelata* in areas remote from this site, but the severity and extent of damage at this site is unique.

Initial observations made with a dissecting microscope were sufficient to confirm the presence of cells of the algal symbiont *Trebouxia* sp. within many of the remaining damaged lichen stems. They were also particularly obvious in small undamaged shoots growing near the holdfast. The transmission electron micrographs in Fig. 5 are consistent with an apparently healthy algal fungal symbiotic relationship and healthy algal cells close to the base of severely damaged specimens. After several days in warm, moist laboratory conditions, there was a marked increase in the algal content of the damaged lichens indicating rapid reproduction of algal cells in favourable conditions.

Results of the photosynthetic pigment analyses carried out on samples taken from the basal 1-2 cm of thalli from control and damaged sites are listed in Table 1. Healthy specimens from the control site contained approximately six times as much chlorophyll and carotenoid pigments as damaged specimens. The ratio of carotenoids to chlorophyll did not vary significantly between the sites. However, the mean chlorophyll a/b ratio was significantly lower for the damaged specimens (3.7 verses 4.6). In all cases, the standard error values are low indicating consistency within each site.

TABLE 1

Chlorophyll and carotenoid content of basal sections of damaged and control samples of U. sphacelata. *Freshly collected samples were extracted and measured in Antarctica*

Pigment Content/Ratio	Damaged *U. sphacelata* (n = 10)	Control *U. sphacelata* (n = 10)
total chlorophyll (ug chl/g dry wt):	43.22 +/- 5.89	266.66 +/- 12.33
chlorophyll a/b ratio:	3.67 +/- 0.21	4.62 +/- 0.10
total carotenoids (ug carot/g dry wt.):	23.75 +/- 2.99	143.95 +/- 7.30
carotenoid/chlorophyll ratio:	0.55 +/- 0.01	0.54 +/- 0.004

Photosynthetic Status of Damaged U. sphacelata

Samples from the damaged lichen site demonstrated an active photosynthetic response to the Plant Stress Meter II probe. The mean initial (Fo), maximum (Fm) and variable fluorescence (Fv) values, and the mean ratio of variable fluorescence to maximum fluorescence (Fv/Fm) of the damaged and control samples are listed with standard errors in Table 2. In comparison with the control samples, the damaged lichen exhibits reduced values of Fo, Fm and Fv, but the Fv/Fm rato is not significantly different (Fv/Fm: 0.422 +/- 0.021 and 0.435 +/- 0.015 respectively).

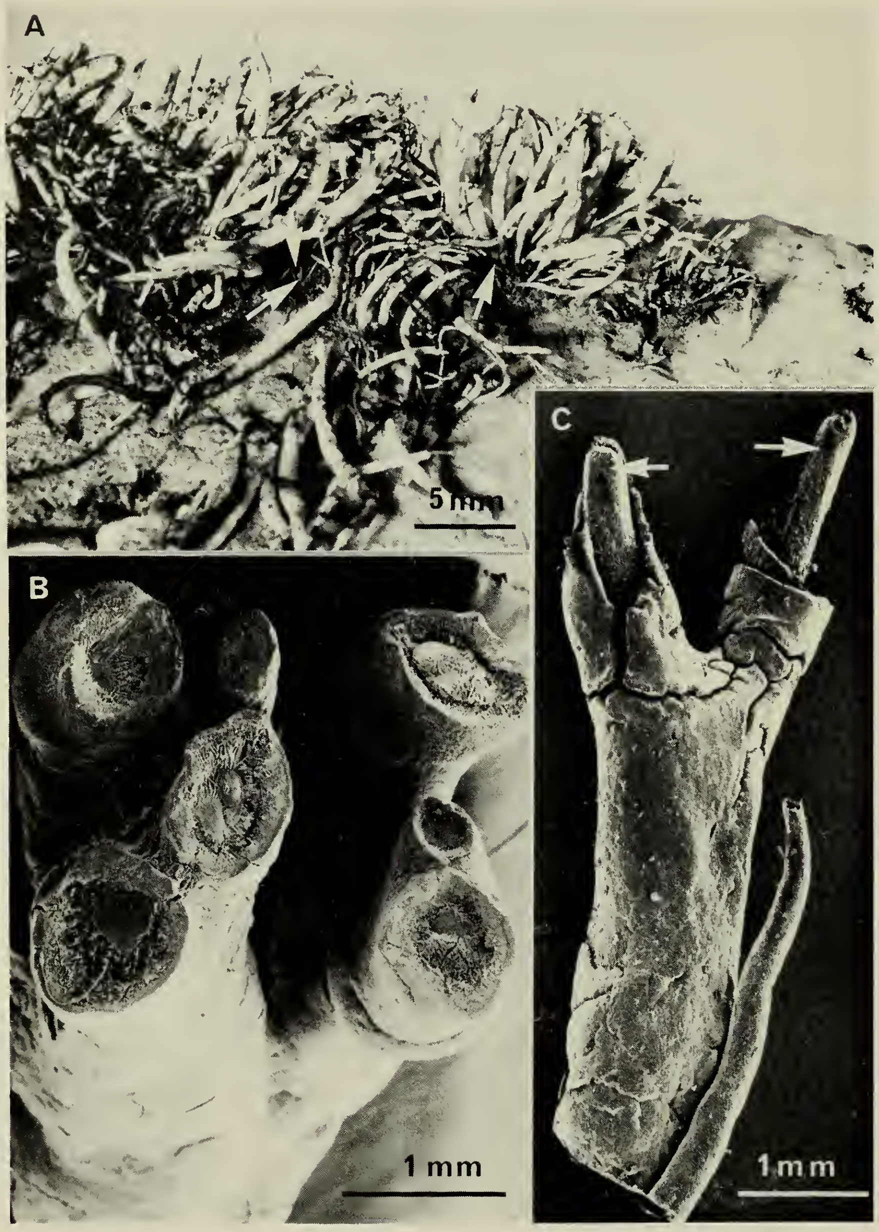

Fig. 4. Damaged *U. sphacelata*; (**A**) remaining basal sections of damaged lichen with numerous small intact shoots, (**B**) SEM showing the clear breaks of naturally 'shaved' thalli, (**C**) SEM of basal section of damaged thallus with central strand protruding.

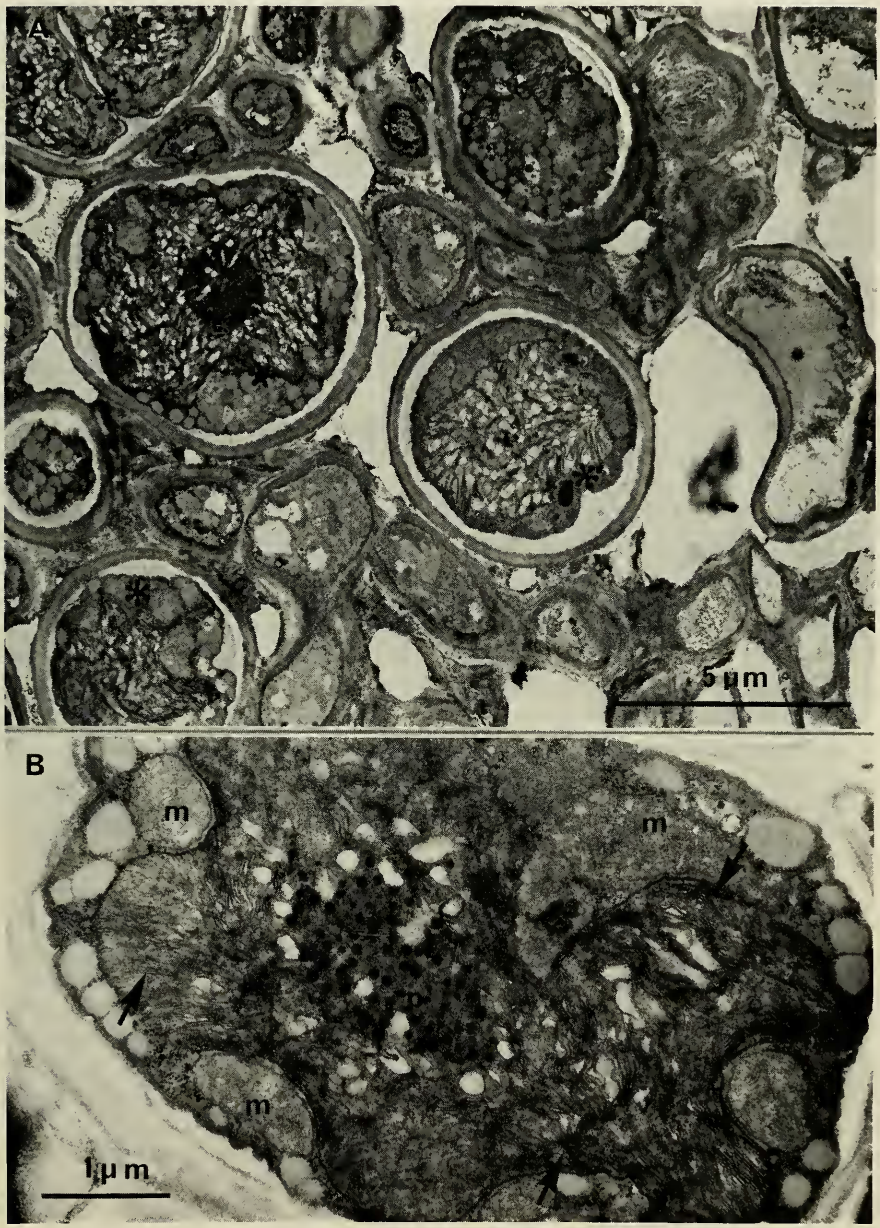

Fig. 5. TEM of damaged *U. sphacelata* showing: (**A**) transverse section of thallus close to the holdfast (phycobiont = asterisk), (**B**) transverse section of algal cell; chloroplast central pyrenoid = p, thylakoids = arrow, and mitochondria = m.

TABLE 2

Chlorophyll fluorescence at 5° C of damaged and control samples of U. sphacelata. *The experiment was performed in Australia on material which had been kept frozen at -20° C*

Fluorescence Values	Damaged *U. sphacelata* (n = 20)	Control *U. sphacelata* (n = 23)
Initial fluorecence (Fo):	0.163 +/- 0.013	0.280 +/- 0.030
Maximum fluorescence (Fm):	0.297 +/- 0.029	0.536 +/- 0.072
Variable fluorescence (Fv):	0.135 +/- 0.018	0.256 +/- 0.043
Variable fluorescence / Maximum fluorescence (Fv/Fm):	0.422 +/- 0.021	0.435 +/- 0.015

DISCUSSION

Chlorophyll fluorescence is a sensitive indicator of the photosynthetic competence of plants and has been widely used to monitor photosynthetic activity in field situations and to register plant responses to stress (Hetherington *et al.*, 1989; Hetherington and Oquist, 1988; Ögren, 1988). Variable fluorescence (Fv) originates from the pigments of photosystem II and is related to the availability of electron accepting quinone molecules. When these are all reduced, fluorescence is maximal (Fm). The ratio of Fv/Fm is proportional to the photochemical efficiency of photosystem II and correlates very well with the quantum yield of net photosynthesis (Oquist and Wass, 1988). An Fv/Fm ratio of around 0.8 is typical for C_3 higher plants under ideal conditions (Bjorkman and Demmig, 1987). Mean Fv/Fm ratios of around 0.4 for *U. sphacelata* from both the control and damaged sites indicates that the photosynthetic efficiency of the algal symbionts in the healthy and in many of the damaged lichens are similar, and that both experienced a similar degree of stress (possibly due to less than optimal hydration conditions) during the experiment reported in Table 2. It is not yet clear why the fluorescence yield with all reaction centres open (Fo) and the variable (Fv) and maximum (Fm) fluorescence values are significantly lower in the damaged thalli. Differences in the physical properties of the mycobiont surrounding the algal cells and the concentration of photoprotective lichen pigments may be relevant.

The presence of healthy cells of the algal symbiont *Trebouxia* sp. in the lower fronds of many of the damaged lichens was confirmed by electron microscopy. The algal cells in the basal 10 mm or so of the damaged remnant thalli were indistinguishable from those of healthy lichens. The essentially identical carotenoid to chlorophyll ratios in lichens from affected and control sites is also consistent with the presence of healthy algal cells in physically damaged specimens. Although the damaged lichens contain normal, photosynthetically competent *Trebouxia*, their mean chlorophyll and carotenoid concentrations are very low compared to those of the lower 1-2 cm sections of control lichens. This is mainly due to a lower concentration of algal cells. We do not however, discount the possibility that the total chlorophyll and carotenoid concentrations per cell are also reduced. A possible explanation for the lower chlorophyll a/b ratio in damaged plants is that it is a response to increased light intensity following loss of the upper heavily pigmented branches of the thallus which normally shade the paler lower regions.

The severity and extent of the damage to lichens described in this study is unique in the Windmill Islands region. Smaller isolated examples of sheared *U. sphacelata* have been observed in areas remote from the station, but the damage has not been severe or widespread, affecting only occasional isolated lichens. In the more distant examples, the shear zone is located well above the holdfast in the black pigmented areas of the upper fronds and the damaged lichens are not so easily distinguished from surrounding healthy material by colour.

Despite the close proximity of these large affected areas to the new station, there is as yet no direct evidence to link the damage directly to events that have occurred due to construction activities or other human impact. The cause of damage is unknown. Since the soil pH in the affected area is low (pH 4) and consistent with the surrounding mean, it is most unlikely that the damage was caused by pollution by alkaline cement dust. In contaminated areas downwind of the concrete batching site the soil is alkaline, with values up to pH 9 (Adamson and Seppelt, 1990). A separate study of alkaline pollution in this area has shown that *U. sphacelata* affected by cement dust pollution is typically bleached, shrivelled and in many cases weakened or detached at the holdfast (Adamson and Seppelt, 1990). These symptoms of damage are quite different to those found in the areas described in this paper.

Disease, toxic fumes and physical abrasion have also been suggested as possible causes of damage. Disease seems unlikely as there is no evidence of viral or bacteriological infection or invasive fungi in the electron-micrographs. A catastrophic leak of poisonous fumes and an inconsistent snow cover could explain the distribution of small scattered healthy patches within the affected areas. Physical abrasion by ice, soil and rock particles carried by a particularly strong wind event may be a natural explanation for the damage consistent with occasional isolated examples of similar damage in other locations and with patchy snow or ice cover protecting some areas within the damaged sites. It is however difficult to explain the sharp boundaries with surrounding healthy material and the existence of two discrete affected areas. There may of course have been a combination of factors resulting in this damage, for example chemical contamination or disease could make the lichens more susceptible to physical abrasion by airborne particles.

The presence of numerous young and still mainly unbranched thalli amongst the damaged remains of a previously flourishing stand of mature *U. sphacelata* indicates that the damaged areas to the south-east of the new station domestic building are now recovering. It also implies that the damage was caused by a catastrophic event and is not symptomatic of an ongoing chronic pollution problem at Casey Station.

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References

ADAMSON, E., and SEPPELT, R. D., 1990. — A comparison of airborne alkaline pollution damage in selected lichens and mosses at Casey Station, Wilkes Land, Antarctica. *In* KERRY, K. R., and HEMPEL, G., (eds), *Antarctic Ecosystems*. Heidelberg: Springer Verlag.

ANDERSON, J. M., and BOARDMAN, N. K., 1964. — Studies on the greening of dark-grown bean plants. II. Development of photochemical activity. *Aust. J. Biol. Sci.* 17: 93-101.

BJORKMAN, O., and DEMMIG, B., 1987 — Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta.* 170: 489-504.

BLIGHT, D. F., and OLIVER, R. L., 1982. — Aspects of the geologic history of the Windmill Islands, Antarctica. *In* CRADDOCK, C., (ed), *Antarctic Geoscience*. Wisconsin: University of Wisconsin Press.

BROUERS, M., and MICHEL-WOLWERTZ, M., 1983. — Estimation of protochlorophyll(ide) contents in plant extracts: re-evaluation of the molar absorption coefficient of protochlorophyll(ide). *Photosyn. Res.* 4: 265-270.

HETHERINGTON, S. E., and OQUIST, G., 1988 — Monitoring chilling injury: a comparison of chlorophyll fluorescence measurements, post chilling growth and visible symptoms of injury in *Zea mays*. *Physiol. Plant* 72: 241-247.

——, HE, J., and SMILLIE, R. M., 1989. — Photoinhibition at low temperature in chilling-sensitive and -resistant plants. *Plant Physiol.* 90: 1609-1615.

HIPKINS, M. F., and BAKER, N. R., 1986. — Spectroscopy. *In* HIPKINS, M. F., and BAKER, M. F., (eds), *Photosynthesis Energy Transduction, a practical approach*. Oxford: IRL Press.

LAWLOR, D. W., 1987 — *Photosynthesis: Metabolism, Control and Physiology*. Essex: Longman Scientific and Technical.

LEWIS-SMITH, R. I., 1986a. — Plant ecological studies in the fellfield ecosystem near Casey Station, Australian Antarctic Territory 1985-1986. *Bull. Br. Antarct. Surv.* 72: 81-91.

——, 1986b. — Report of biological programme at Casey Station, October 1985 to January 1986 (including appendices on personal observations on environmental impact and related matters and general comments on ANARE research). Report to the Australian Antarctic Division, British Antarctic Survey and Australan Antarctic Science Advisory Comittee (unpubl.).

——, 1988. — Classification and ordination of cryptogramic communities in Wilkes Land, Continental Antarctica. *Vegetatio.* 76: 155-166.

ÖGREN, E., 1988. — Photoinhibition of photosynthesis in willow leaves under field conditions. *Planta* 175: 229-236.

OQUIST, G., and WASS, R., 1988. — A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. *Physiol Plant* 73: 211-217.

PARSONS, T. R., MAITA, Y., and LALLI, C. M., 1984. — *A Manual of Chemical and Biological Methods for Seawater Analysis*. Oxford: Pergamon Press.